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THE EFFECT OF WEEKLY STANDARD DOSES OF PRIMAQUINE AND CHLOROQUINE ON G6PD DEFICIENT CAUCASIANS

Final Technical Report

By

E. Salvidio, I. Pannacciulli, and A. Tizianello

March 16, 1968

EUROPEAN RESEARCH OFFICE

United States Army
Frankfurt, Germany

Contract Number DAJA37-67-C-0450

Istituto di Patologia Medica
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ABSTRACT

Daily doses of 30 mg of primaquine cause severe hemolysis in G6PD deficient Caucasians. Effective weekly dosages of 45 mg of primaquine and 300 mg of chloroquine do not cause significant hemolysis in G6PD deficient Negroes. In order to investigate the upper limits of safety to primaquine, the effect of this standard weekly dose on G6PD deficient Caucasians was studies. Seven sensitive Sardinians had significant hemolytic crises after a single weekly administration of the drugs. There was a drop in the hematocrit, a shortened survival of 51Cr tagged red cells, with an average destruction of 400 ml of red cells. G6PD deficient 51Cr tagged red cells belonging to some of these subjects transfused into normals, receiving thereafter weekly doses of primaquine, were rapidly destroyed, convalidating the sensitivity to primaquine. Three G6PD deficient Sardinians did not show any hemolysis after ingestion of weekly doses of primaquine. Their red cells, however, transfused into normals, who received standard weekly doses of antimalarial drugs, were also rapidly destroyed. Investigations should establish if environmental factors (faulty absorption of primaquine, different metabolic breakdown of the drug) or metabolic differences in the G6PD deficient red cells are responsible for this peculiar behavior. As far as the protective role of SH donor drugs is concerned, preliminary clinical studies have failed to show any significant protective action of these drugs.

The apparent *in vitro* protective obtained by preincubating normal intact red cells with cysteine or cysteamine prior to the exposure of the cells to NEM, could possibly be ascribed to chemical reactions between the two compounds.

Aims

The antimalarial action of primaquine is well-known: daily doses of 30 mg have a unique ability in preventing and treating malaria. This therapeutic action has usually no side effects, with the exception of Negroes and Caucasians with red cell G6PD deficiency. In G6PD deficient Negroes the daily administration of 30 or 15 mg of primaquine induces a self-limited hemolytic crisis (1). In G6PD deficient Caucasians the problem is somewhat different: the enzymatic defect of the red cells being more severe, the daily administration of 30 mg of primaquine induces serious hemolytic episodes in which old and young red cells are destroyed. (2) The hemolytic ability of primaquine on G6PD deficient Negroes has stimulated some investigators to study if weekly dosages of the antimalarial drug could nullify its hemolytic side effect. On this assumption Alving and associates (3) have worked out a therapeutic scheme of weekly dosages of 45 mg of primaquine and 300 mg of chloroquine which, while still preserving an effective antimalarial action, had little if any hemolytic effect on G6PD deficient Negroes. The absence of clinical signs of hemolysis when using the above-mentioned doses of primaquine and chloroquine has been confirmed by Ziai and Bowmann on Iranians. (4) Their study, however, was based essentially on the determination of the hematocrit level on a monthly basis, and on the presence or absence of evident clinical signs of hemolysis.

It was thought worthwhile, therefore, to undertake an investigation on the action of weekly dosages of primaquine and chloroquine on G6PD deficient Sardinian males who, as already stated, have an extremely severe deficiency of the red cell enzyme, and whose greater susceptibility to antimalarial drugs has already been ascertained.

In "vivo" studies on the possible protective effects of sulphydril donor drugs or EDTA on sensitive Sardinians and on G6PD deficient red cells transfused into normal recipients who received thereafter antimalarial drugs, were also undertaken. Collateral investigations on the action of these protective drugs on NEM damaged normal red cells "in vitro" were performed in order to better understand, if possible, the relationship between oxidizing drugs, normal or G6PD deficient red cells, and SH donor compounds.

Methods and Materials

Laboratory studies

Hematocrit, hemoglobin and blood group determinations were performed according to standard procedures.

Red blood cells were tagged with radioactive sodium chromate according to the technique of Mollison and Veall. (5) The radioactivity of the blood samples was measured in a well scintillation counter.

Erythrocytic G6PD was measured according to the method of Kornberg and Horecker. (6) SH groups were determined by the method of Beutler and ass. (11) G6P Na salt, NADP Na salts were preparations of Boehringer & Co.

NEM and DTT (Clealand's reagent) were prepared by K & K Lab.

L-cysteine and cysteamine were prepared by FLUKA.

EDTA was prepared by C. Erba, Milan.

Clinical studies

(1) Ten Sardinian males with G6PD deficiency of their red cells, and four Sardinian males without red cells abnormalities volunteered for these studies. Their red cells were tagged with 51Cr and after the baseline assessment of the red cell survival, both normal controls and G6PD deficient Sardinians received one or more weekly doses of 45 mg of primaquine and 300 mg of chloroquine. These were the standard doses used throughout this study if not otherwise stated.

(2) G6PD deficient red cells belonging to 9 Sardinian males, tagged with 51Cr, were transferred into normal subjects with compatible blood groups. The recipients received thereafter, weekly dosages of primaquine and chloroquine (45 mg and 300 mg) once the baseline survival of the transfused G6PD deficient red cells had been assessed.

(3) Normal recipients, in whom 51Cr tagged, G6PD deficient red cells had been transfused, received before, during, and after the weekly administration of the antimalarial drugs, l-cysteine, cysteamine or EDTA.

L-cysteine was administered in daily oral doses of 1 gm Cysteamine was given parenterally in daily doses of 600 mg. EDTA was administered intravenously in daily doses of 2 gm.

Results

Clinical Studies

(1) Three normal Sardinians, without red cell G6PD deficiency, received 3 weekly dosages of primaquine and chloroquine (45 and 300 mg respectively). No hemolysis was observed. The survival of their 51Cr tagged red cells was 28, 22, and 27 days (fig. 1).

(2) Seven out ten G6PD deficient Sardinian males, treated with the standard dosage of antimalarial drugs, had definite hemolysis, which could be evaluated both by 51Cr tagged red cell survival and change in the level of the hematocrit (fig. 2 - 3 - 4 - 5 - 6 - 7 - 8). In only one case (C. A.) the disappearance of the 51Cr tegged red cells from the circulation was not accompanied by significant changes in the level of the hematocrit (fig. 5). In these seven cases the primaquine-chloroquine preparation was administered only once in three cases, twice in three cases, and three times in one case.

(3) In three G6PD deficient Sardinian males repeated weekly administrations of primaquine-chloroquine (45 mg and 300 mg) didn't produce any red cell destruction which could be evaluated by changes in hematocrit or 51Cr tagged red cell sur

vival (fig. 9 - 10 - 11).

(4) In the seven Sardinian males who developed hemolysis, the mean survival of the 51Cr tagged red cells before the administration of the antimalarial drugs, was 21.8 days (table 1). Is only one case the 51Cr T/2 was 12 days although the hematocrit level was normal. After the administration of the primaquine-chloroquine preparation (45 mg and 300 mg), the mean 51Cr T/2 of all seven cases was 8.7 days. The mean percentage of all red cells hemolyzed was 19 percent if calculated on the hematocrit levels, and 24 percent if calculated on 51Cr survival data. The amount of red cells destroyed in the hemolytic crisis in three cases in which the red cell mass could be calculated was 450 ml (S.A.), 390 ml (F.U.), and 350 ml (M.P.). One G6PD deficient Sardinian male (F.U.) received twice a single weekly dose of primaquine-chloroquine (45 mg and 300 mg) (fig. 7), the first dose being administered together with EDTA intravenously, the second one with l-cysteine orally. Each time the antimalarial drugs have caused hemolysis with a 51Cr half life, compared to that of the other susceptible cases receiving the same dosages of antimalarial drugs.

(5) G6PD deficient red cells to be transfused into normal recipients who received primaquine, were drawn from sensitive Sardinian males belonging to the following groups: a) G6PD deficient Sardinian males who were susceptible to the drug (point 2); b) G6PD deficient subjects who did not show any hemolysis after receiving the weekly dosage of primaquine (point 3); c) G6PD deficient subjects who were not treated with primaquine.

In all normal recipients (table 2, fig. 12) the administration of the primaquine chloroquine preparation caused a clear-cut shortening of the survival of the transfused, sensitive red cells. Mean red cell survival during the baseline period was 21.2 days; after administration it decreased to a mean value of 7.1 days. After a mean period of hemolysis of 4 days, the survival of the remaining tagged sensitive cells was normal or even longer than normal, probably owing to the destruction of the older cells. The mean percentage of the red cells hemolyzed was 31 percent.

(6) G6PD deficient red cells were transfused into normal recipients who received EDTA, cysteine or cysteamine, before and during the administration of the standard dose of antimalarial drugs. No clear-cut modification of the hemolytic action of primaquine could be observed in these cases (fig. 9 and 12).

(7) Normal red cells were damaged with BMHP-Hg^{197} (which inhibits the GSH groups of the red cell surface) and transfused into the normal donors, some of whom received intravenous infusions of two grams of Vit. C. The infusions were begun twenty minutes before the injection of the damaged cells, and lasted for at least an hour. No difference in the half life of the damaged cells, with or without the administration of Vit. C. could be observed.

Experimental Studies

(1) Investigations by Kirkman, Chung, and ass. (7 - 8) have demonstrated that sulphydril inhibitors inhibit also G6PD. It was assumed therefore, that by treating normal red cells with NEM, it is possible to obtain red cells similar to G6PD deficient cells treated with oxidative drugs. The possible protective role of some SH-donor drugs (DTT, cysteine, cysteamine, and GSH) on such cells was studied in vitro. The following results were obtained:

a) NEM inhibits G6PD activity both in hemolysates and in intact normal red cells. (table 3)

b) Preincubation of red cell hemolysates with DTT, cysteine, or GSH preserves the G6PD activity from NEM's inhibitory action. (table 4) On the contrary, once the inhibition of G6PD activity by NEM was established, DTT was unable to restore the enzymatic activity. (table 5)

c) Preincubation of normal intact red cells with cysteine prevented the inhibitory action of NEM. On the contrary, once the inhibition by NEM of G6PD activity of the intact normal cells was established, the subsequent addition of cysteine or cysteamine was unable to restore the enzymatic activity of the cells. (table 6)

(2) Red cells of normal rats incubated with NEM and tagged with 51Cr, were transfused into the animals, a group of which was previously injected with 500 mg of Vit. C or 50 mg of cysteine. After 20 hours the rats were sacrificed and the radioactivity of the spleen, the liver, heart, lungs, and carcass was measured. Preliminary results do not show significant differences in the distribution of radioactivity between treated and untreated animals.

Discussion

These studies suggest that in the greater part of G6PD deficient Caucasians, the administration of single weekly dosages of 45 mg of primaquine and 300 mg of chloroquine induces destruction of red cells, with changes in the hematocrit level, bilirubinemia, and reticulocyte number.

The severity of the hemolytic crisis differs among the sensitive subjects: some of them showed a marked hemolysis with a sensible drop in the hematocrit levels. In three cases the amount of the red cell mass destroyed has been calculated to be about 400 ml.

Transfusions of G6PD deficient red cells, belonging to this group of subjects or to other sensitive Caucasians, into normal recipients receiving thereafter one or more single weekly doses of primaquine, have been performed. The susceptibility to hemolysis of Caucasians to standard weekly doses of antimalarial drugs was fully convalidated also from a quantitative point of view. In some recipients repeated administrations of weekly doses of 45 mg of primaquine caused red cell destruction at each ingestion.

Weekly doses of primaquine didn't cause any change in survival of 51Cr tagged red cells or in hematocrit levels in three aot of ten G6PD deficient Caucasian males. The peculiar behavior of these three subjects may be possibly related to: a) faulty absorption of the single dose of primaquine; b) different metabolic breakdown of the drug; c) a different metabolic pattern of the G6PD deficient red cells of these subjects. The red cells of two of these same subjects, when transfused into normal recipients who received 45 or 60 mg of primaquine per week, underwent a significant destruction. This fact would suggest the possible existence of environmental factors rather than metabolic differences of the red cells.

Investigations are in progress in order to possibly elucidate this problem.

The results here reported are similar to those described by George and ass. (9) These authors have described hemolytic crises following the ingestion of a single 45 mg tablet of primaquine in two G6PD deficient Caucasians. Two other Caucasians had ingested primaquine in weekly dosages for some months without developing hemolytic crises. These two latter cases seemed to behave like our G6PD deficient Sardinians who received several doses of primaquine without developing hemolysis.

As far as the possible protective role of SH donor drugs is concerned, preliminary clinical studies have failed to show any significant protective action of these drugs.

The apparent "in vitro" protective effect obtained by incubating normal intact red cells with cysteine or cysteamine prior to the exposure of the cells to NEM, could be ascribed to chemical reactions between the two compounds. (10)

TABLE 2

Studies with 51Cr tagged red cells belonging to G6PD deficient Sardinians, transfused into normal recipients

No.	Cases		Baseline 51Cr T/2 (days)	51Cr T/2 after primaquine (days)	Duration of hemolysis (days)	R.b.c. hemolyzed percent calculated from	
						51Cr T/2	Ht level
1	1a		16	6	5	31	19.5
2	1b		28	10	-	-	-
3	3a		16	3	3	46	15
4	3b		22	9	-	-	-
5	3c		16.5	7	-	-	-
6	6a		17	7	4	21	n.d.
7	6b		18	9	-	-	-
8	7a		22.5	1.5	2	68	48
9	7b		23	3.5	4	45	n.d.
10	8a °		-	-	-	-	-
11	8b		17	10.5	9	31	n.d.
12	8c		20	9	-	-	-
13	10a		23	5.5	3	25	40
14	L.E.	a	16	4	2	8	n.d.
15		b	26.5	10	5	18	40
16		c	22.5	9.5	4	16	48
17	C.G.	a	21	10.5	-	-	-
18		b	21	3	3	48	n.d.
19		c	34	10	4	20	24

° 30 mg twice

TABLE 1

^{51}Cr red cell survival studies in ten G6PD deficient Sardinians

No.	Name	Baseline ^{51}Cr T/2 (days)	^{51}Cr after primaquine (days)	Duration of hemolysis (days)	R.b.c. hemolyzed percent calculated from	
					^{51}Cr T/2	Ht level
1	C.P.	29	10.5	9	33	16
2	P. G. M.	18	10	-	-	-
3	S. A.	24	7	6	30	29
4	C. A.	28	11.5	-	-	-
5	F. G.	12	7.5	-	-	20
6a	F. U.	19	7.5	4	20	15
6b	"	-	7	2	18	-
7	M. P.	23	7	3	19	16
8	G. L.	24	24	-	-	-
9	C. I.	21	21	-	-	-
10	N. G. A.	21	21	-	-	-

TABLE 3

G6PD activity in normal r.b.c. hemolysates and in intact (°) normal r.b.c. after incubation with different concentrations of NEM

NEM µm/ml	G6PD activity percent hemolysate	G6PD activity percent intact r.b.c.
0	100	100
0.05	82	/
0.1	72	/
0.25	19	/
0.50	11	78
1.00	0	65
2.00	/	30
5.00	/	0
10.00	/	0

(°) after incubation of the intact red cells with NEM at 37°C for 17 m', activity was determined on the hemolysate

TABLE 4

G6PD activity in normal r.b.c. hemolysates incubated with 0.5 um/ml of Nem, and with DTT or cysteamine in various concentrations

DTT or cysteamine concentrations µm/ml	G6PD activity percent in the presence of DTT	G6PD activity percent in the presence of cysteamine
0.5	88.5	43
1	/	55
2	88.5	61
5	101.9	75
10	97.1	100
50	/	80

TABLE 5

G6PD activity of normal red cell hemolysates, preincubated with NEM or DTT, followed by the incubation of DTT or NEM

Preincubation with:	G6PD activity percent	Incubation with:	G6PD activity percent
NEM 0.5 µm/ml	0.5	DTT 5 um/ml	0.4
DTT 5 µm/ml	101	NEM 0.5 µm/ml	98

TABLE 6

G6PD activity of normal intact r.b. cells preincubated with NEM or cysteine (at various concentrations), followed by incubation with cysteine or NEM

Preincubation with:	G6PD activity Percent	Incubation with:	G6PD activity percent
intact r.b.c. + NEM 5 µm/ml	0.0	+Cysteine 50 µm/ml	0
intact r.b.c. + cysteine 10 um/ml	102	+NEM 5 µm/ml	16
intact r.b.c. + cysteine 15 um/ml	109	+NEM 5 µm/ml	30
intact r.b.c. + cysteine 30 um/ml	112	+NEM 5µm/ml	70
intact r.b.c. + cysteine 50 µm/ml	120	+NEM 5µm/ml	123

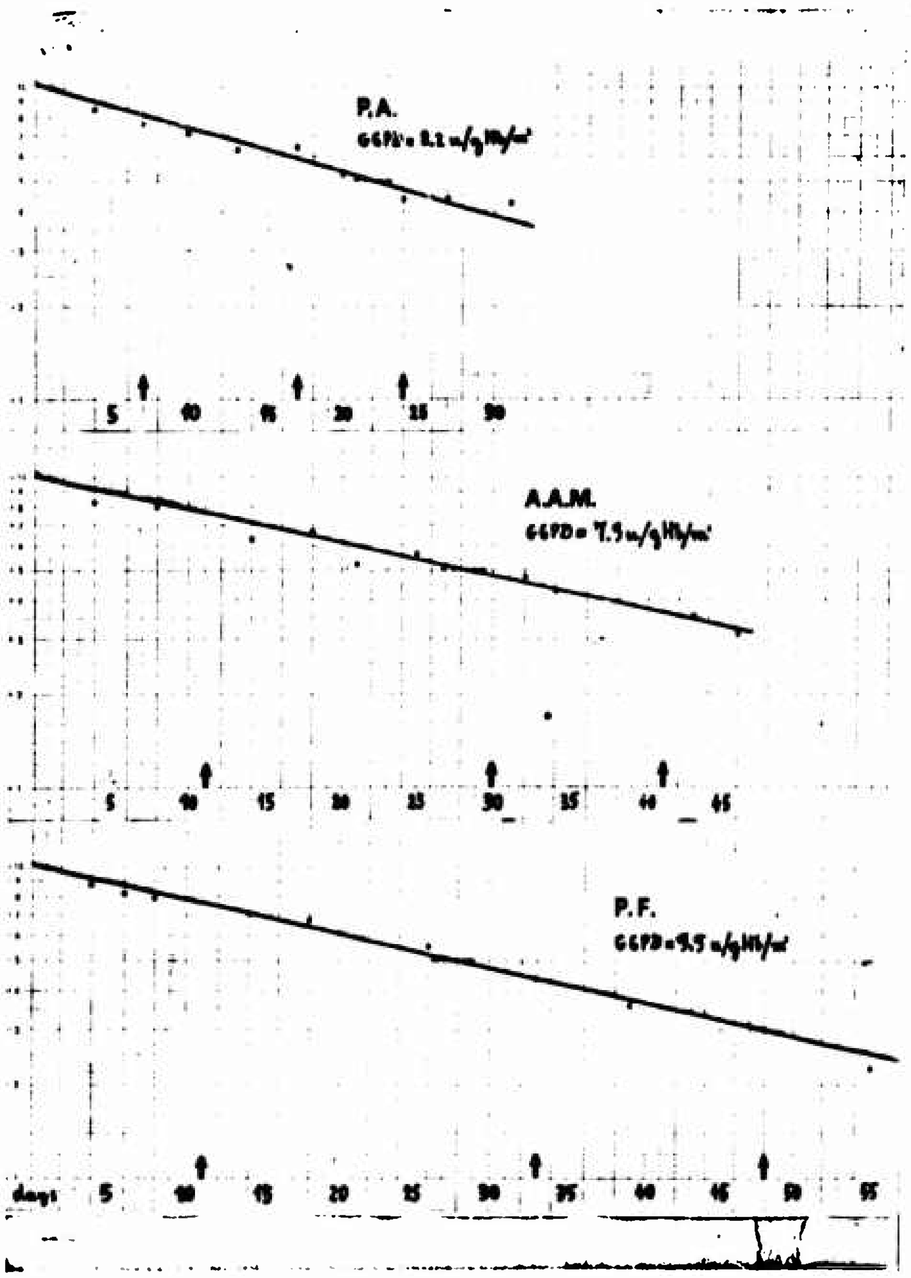


Fig. 1

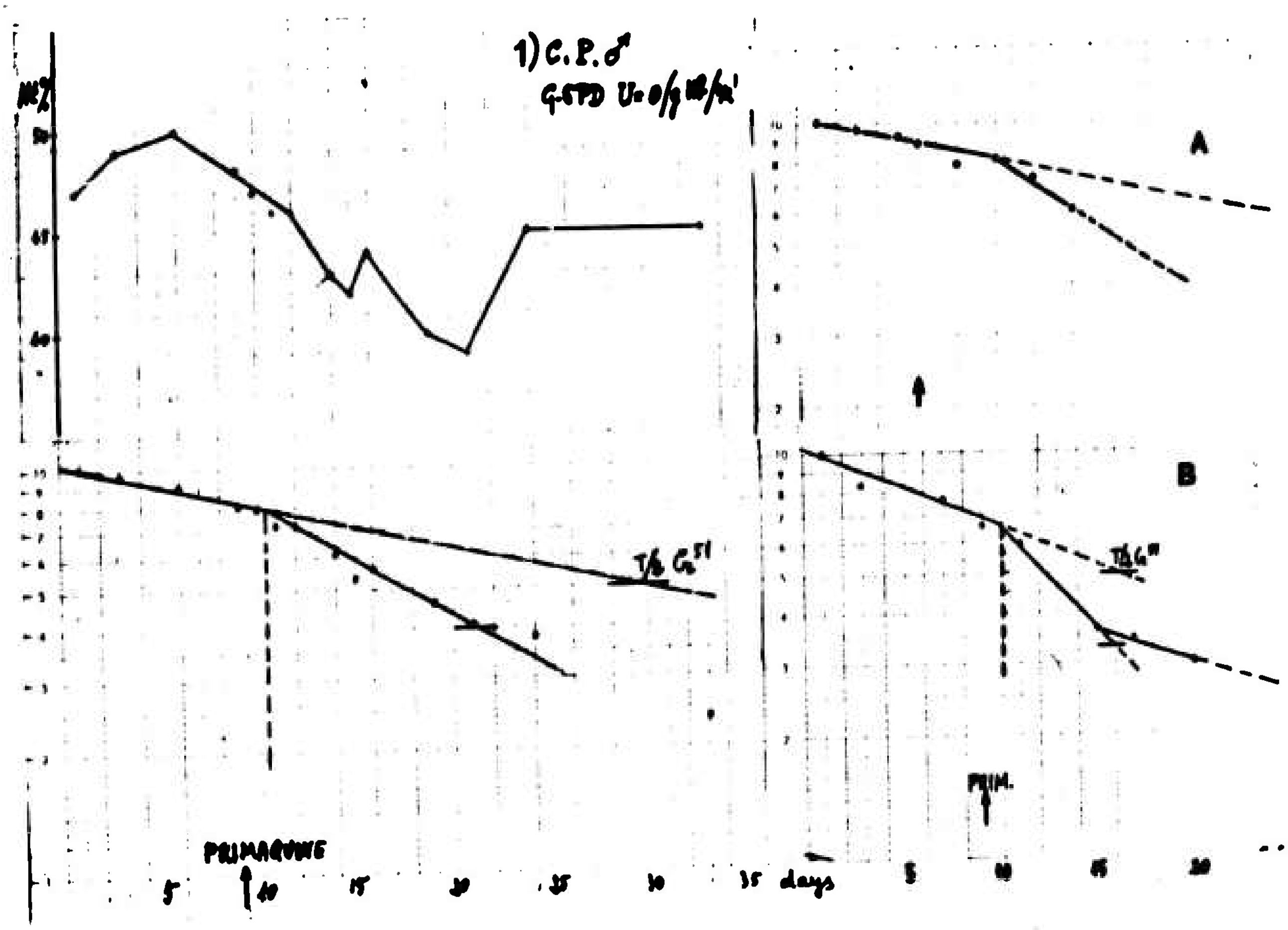
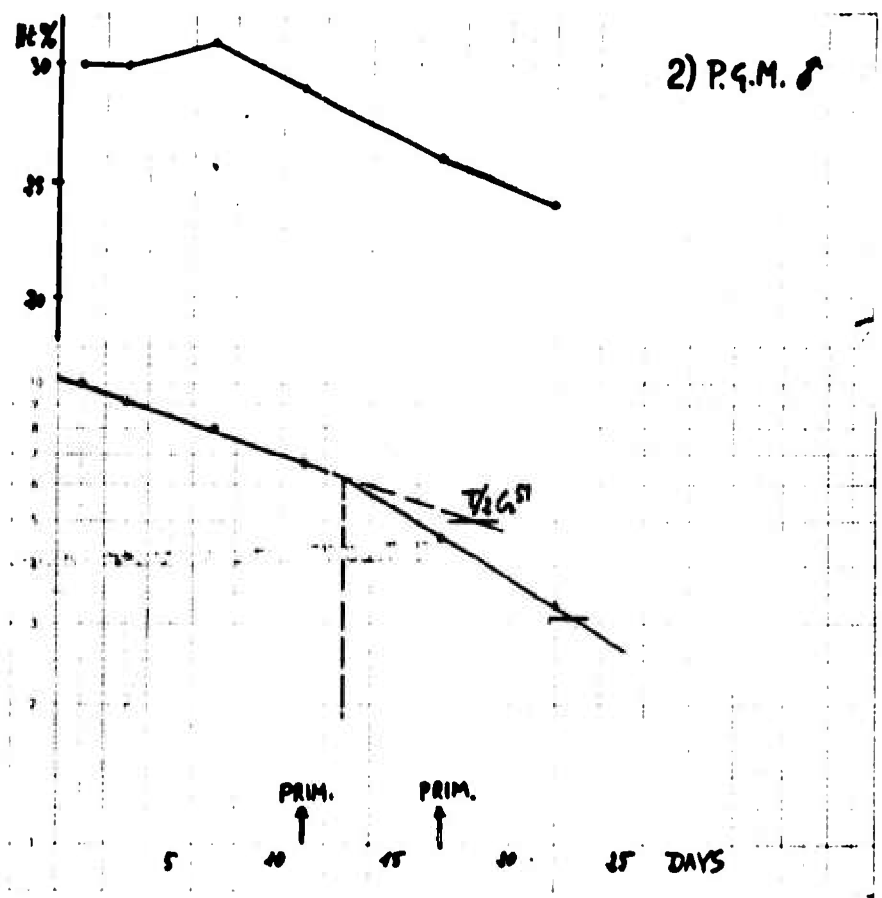


Fig. 2



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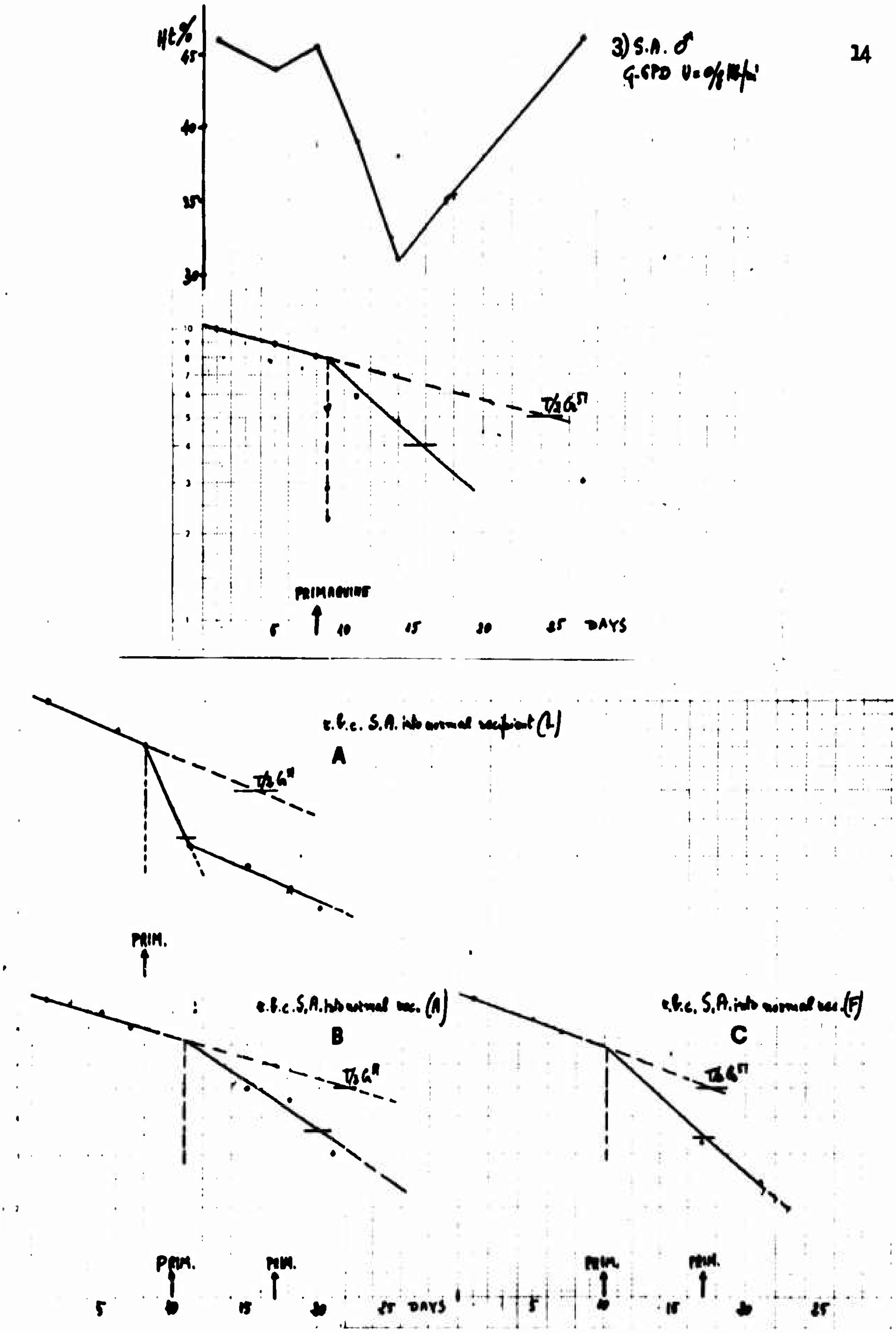

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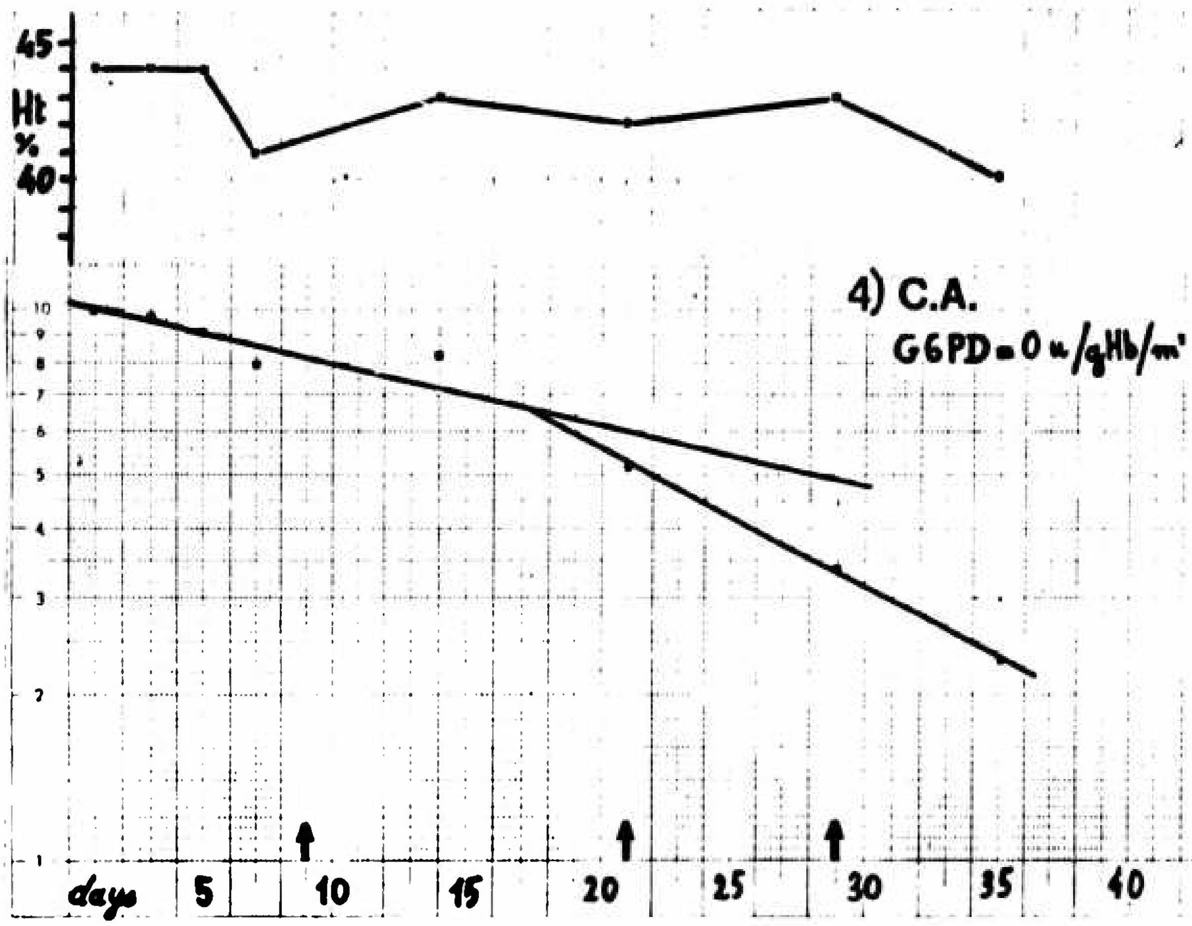

Fig. 5

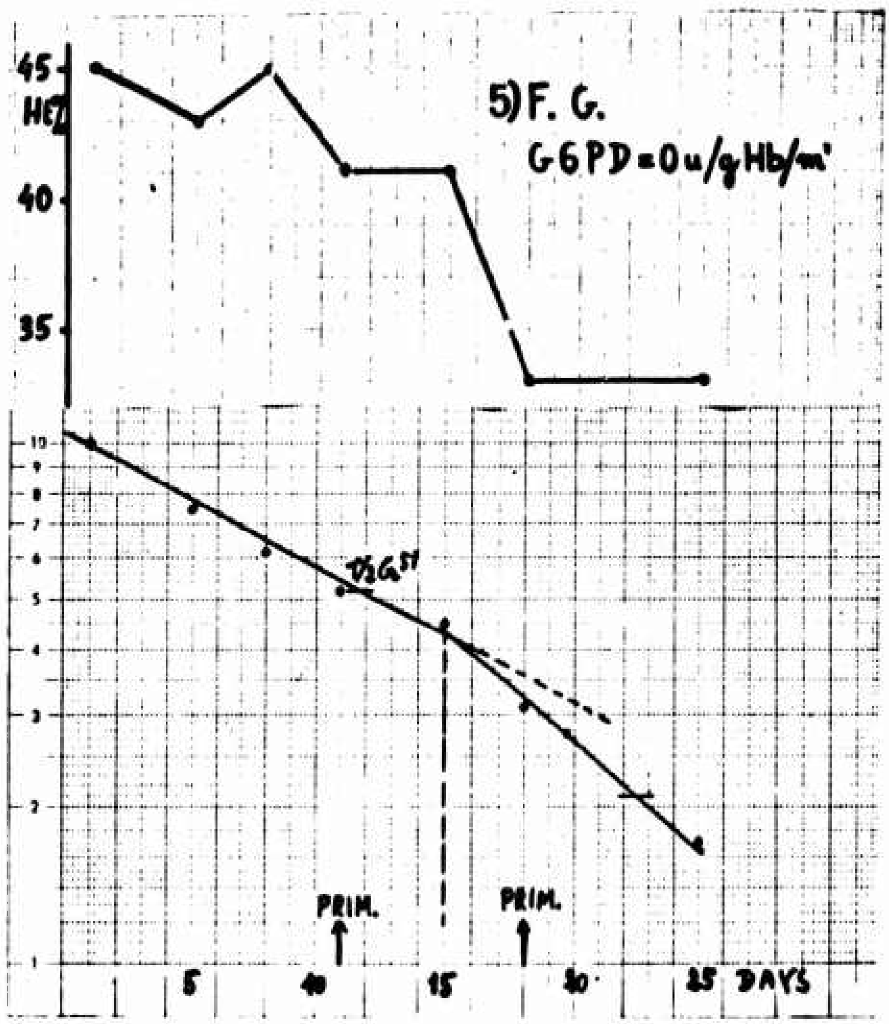
45
HE%
40
35
5) F. G.
G6PD=0u/gHb/m'
10
9
8
7
6
5
4
3
2
1
T½Cr51
PRIM.
PRIM.
5
10
15
20
25 DAYS

Fig.6

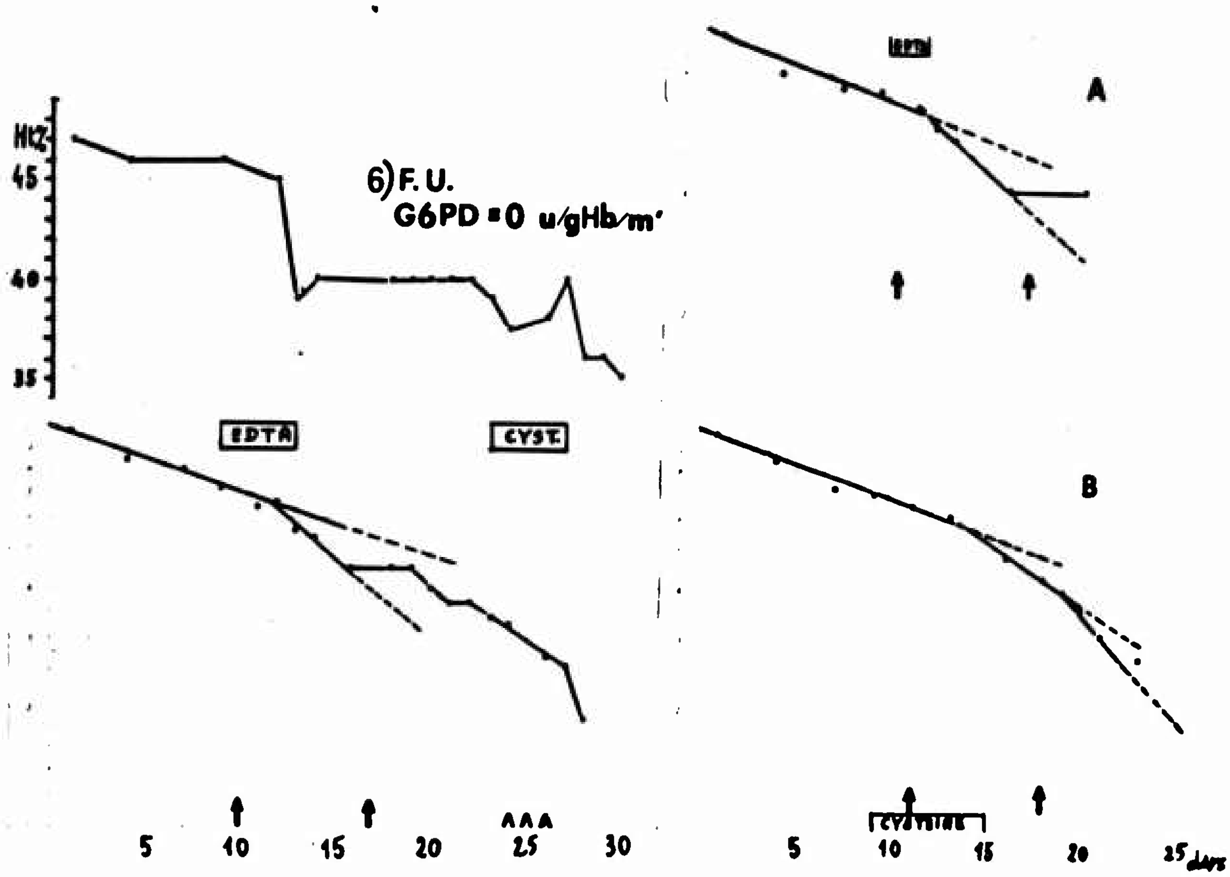
Htz
45
40
35
6) F. U.
G6PD = 0 u/gHb/m'
EDTA
CYST.
A
B
5
10
15
20
25
30
5
10
15
20
25 days

Fig.7

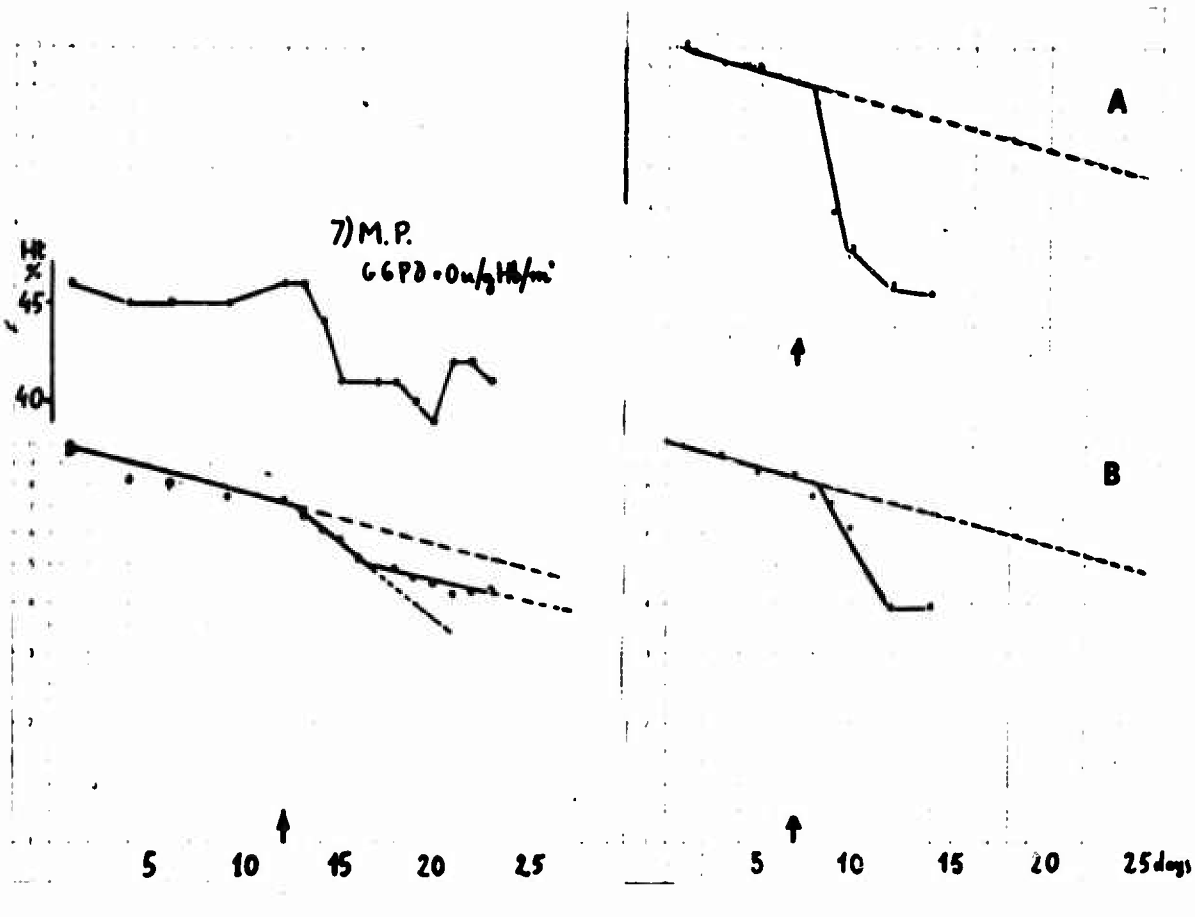

Fig. 8

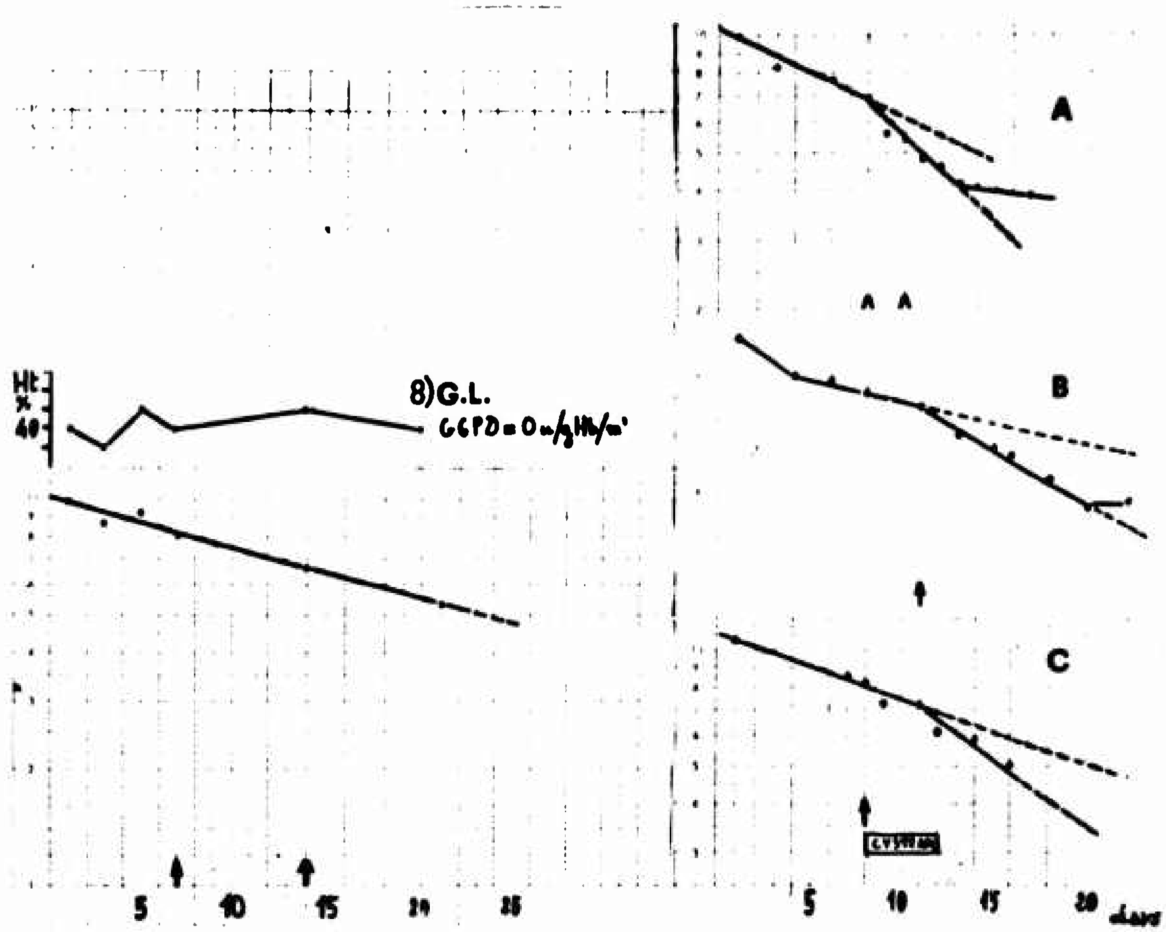

Fig. 9

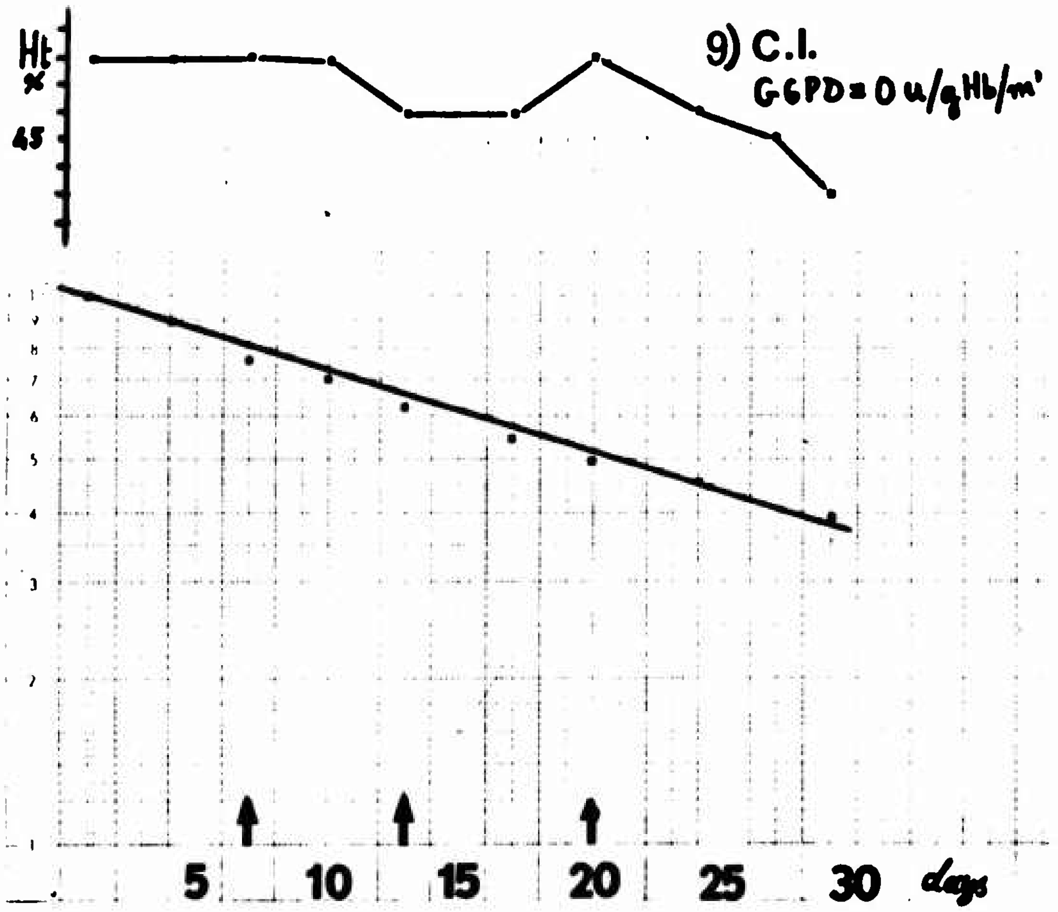

Fig. 10

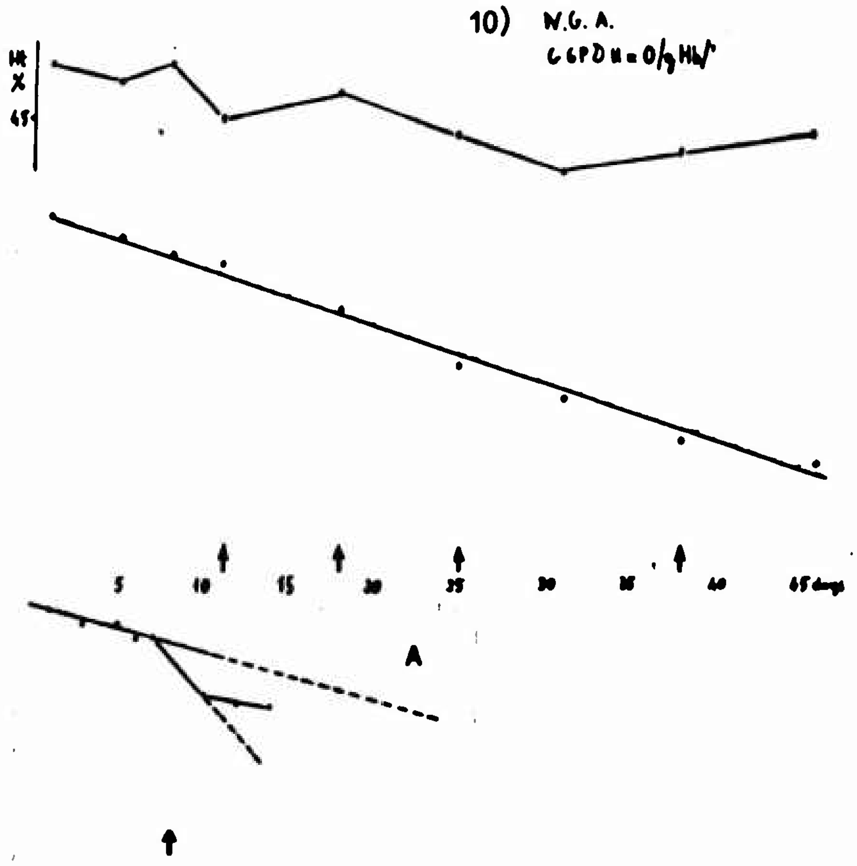

Fig. 11

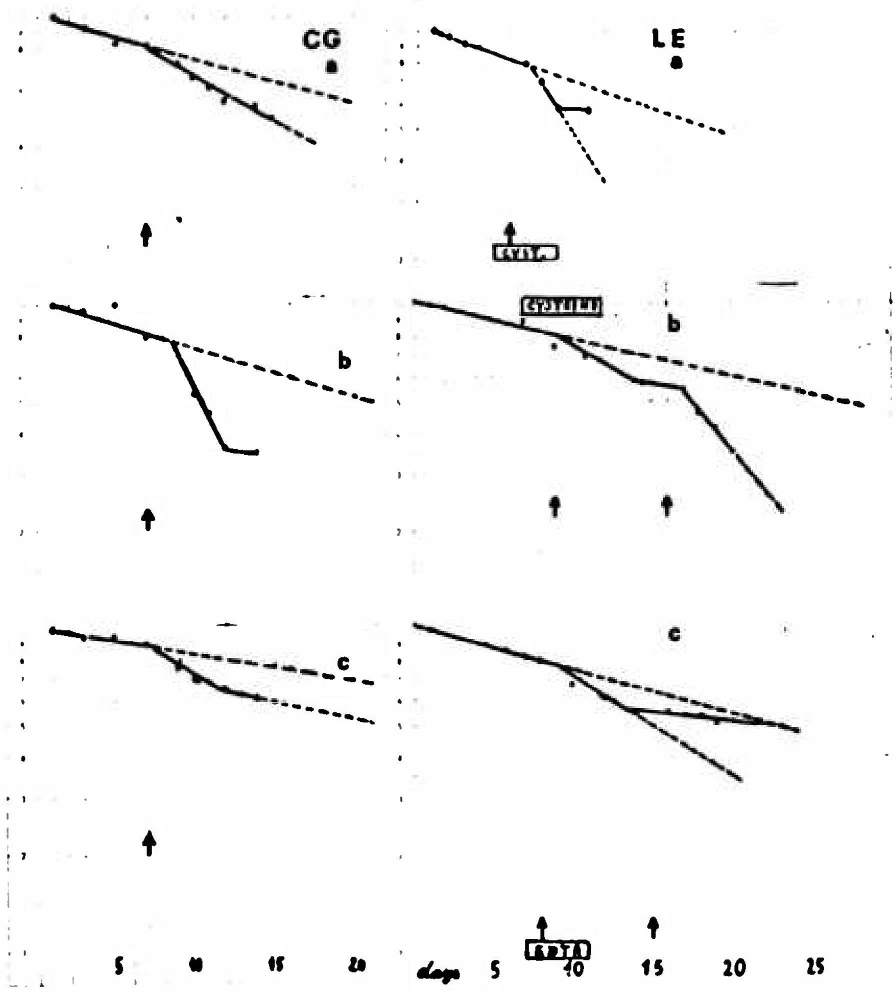

Fig. 12

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13. ABSTRACT In order to investigate the upper limits of safety to primaquine, the effect of weekly standard doses of 45 mg of primaquine and 300 mg of chloroquine on G6PD deficient Caucasians was studied. Seven sensitive Caucasians had significant hemolysis after a single weekly dose of the drugs measured by the change in the 51Cr tagged red cell survival and in the hematocrit levels. G6PD deficient 51Cr tagged red cells belonging to some of these subjects transfused into normals, receiving thereafter weekly doses of primaquine, were rapidly destroyed. Three G6PD deficient Sardinians did not show any hemolysis after ingestion of weekly doses of primaquine. Their red cells, however, transfused into normals, who received weekly standard doses of primaquine, were also rapidly destroyed. Investigations should establish if environmental factors (faulty absorption of primaquine, different metabolic breakdown of the drug) or metabolic differences in the G6PD deficient red cells are responsible for this peculiar behavior. As far as the protective role of SH donor drugs is concerned, preliminary clinical studies have failed to show any significant protective action of these drugs. The apparent *in vitro* protection obtained by preincubating normal *intact* red cells with cysteine or cysteamine prior to the exposure of the cells to NEM, could possibly be ascribed to chemical reactions between the two compounds.

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Hemolysis						
Glucose-6-Phosphate Dehydrogenase Deficiency						
Primaquine Sensitivity in Caucasians						
Pharmacogenetics						

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